Prevalence of and Risk Factors for *Listeria* Species on Dairy Farms

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ABSTRACT

This cross-sectional study determined the prevalence of *Listeria* spp. in bulk-tank milk on dairy farms in the region of Galicia in northwest Spain. The aim was to identify management practices associated with the presence of *Listeria* spp. and possible effects on milk hygienic quality. A total of 98 farms was randomly selected on the basis of an expected prevalence of 6.5% for *Listeria monocytogenes* from 20,107 dairy farms in the region. Bulk-tank milk samples were obtained from 98 farms, fecal samples from lactating cows from 97 farms, and silage samples from 83 farms. *Listeria monocytogenes* was detected in 6.1, 9.3, and 6.0% of these samples, respectively. Statistical analyses confirmed the relationship between low silage quality (as indicated by high pH) and presence of *Listeria* spp. in silage (29.5 vs. 6.2% for pH above or below 4.5, respectively). Only milking system [tie-stall systems (28.6%) vs. parlor milking (10%)] and inadequately controlled milking order [yes (32.0%) vs. no (10.7%)] had statistically significant effects on management practices for increasing the risk of *Listeria* contamination of bulk-tank milk.

Key words: *Listeria* spp., prevalence, risk factor, dairy farm

INTRODUCTION

The *Listeria* species are gram-positive, motile, intracellular, facultative anaerobes (Cooper and Walker, 1998; Winter et al., 2004). The genus includes *Listeria monocytogenes*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, *Listeria ivanovii* (ssp. *ivanovii* and *londoniensis*); the taxonomic status of *Listeria grayi*, *Listeria murrayi*, and *Listeria denitrificans* is uncertain (Vázquez-Boland et al., 2001).

Listeria monocytogenes is included in the World Health Organization list of foodborne pathogens (WHO, 2002), and is the most important *Listeria* species in terms of public health risk and frequency of appearance

in foodstuffs (Fenlon and Wilson, 1989). *Listeria seeligeri* and *L. ivanovii* may be pathogenic in humans (Wiedmann, 2003).

Listeria monocytogenes is ubiquitously present in diverse environments including water, grass, silage, decomposing organic matter, soil, and feces (Hassan et al., 2001). It has a high capacity for persistence under adverse conditions, surviving at refrigeration temperatures and over a wide range of pH (Low and Donachie, 1997). Both humans and diverse wild and domestic animals may be asymptomatic carriers of *L. monocytogenes*, which is widely present in the feces of cattle, pigs, chickens, turkeys, ducks, crustaceans, and flies. Similarly, cattle may shed these bacteria in milk over long periods without showing any symptoms of disease (Low and Donachie, 1997). *Listeria monocytogenes* can be pathogenic for animals, and the development of clinical listeriosis was associated with stress (Cooper and Walker, 1998). The principal source of infection for ruminants is spoiled silage (Low and Donachie, 1997; Vázquez-Boland et al., 2001; Wiedmann, 2003), although it may come from contaminated water or feed contaminated by avian or insect vectors (Cooper and Walker, 1998).

Listeria monocytogenes may reach bulk tanks as a result of exogenous contamination via the milking equipment, because of fecal contamination during milking, or, less frequently, by an intramammary route following generalized asymptomatic infection or mastitis (Cooper and Walker, 1998; Hassan et al., 2001; Winter et al., 2004).

The aims of the present study were to determine the prevalence of *Listeria* species in on-farm bulk tanks, to identify management practices associated with the presence of *Listeria* species in bulk tanks, and to assess the possible influence of *Listeria* species on milk hygienic quality.

MATERIALS AND METHODS

Study Design

This was a cross-sectional study carried out in 2005. Ninety-eight dairy farms were randomly selected in Galicia (northwest Spain), a region accounting for 40% of Spain's total milk production. The number of farms in-

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cluded was calculated from 20,107 dairy farms in Galicia (Instituto Galego de Estatística, 2005) using the following assumptions: 1) the expected prevalence of *L. monocytogenes* was 6.5% (Van Kessel et al., 2004), 2) the level of confidence was 95%, and 3) the acceptable level of error in the prevalence estimate was 5%. All calculations were done with the software package WINEPI (Carmelo Ortega, Zaragoza, Spain).

The mean number of animals per herd was 61.3 (8 to 480, SD = 67.1), and the mean number of lactating cows was 35.0 (4 to 260, SD = 39.3). Of the 98 farms, 41.2% had free-stall housing with a milking parlor, and the remaining 58.8% had tie-stall housing with pipeline (43.3%) or bucket (15.5%) milking.

Data Collection: Questionnaire

Each farmer was interviewed on the farm at the time of sample collection and pH determination using a questionnaire about the following farm characteristics and aspects of management; however, some of these aspects were observed directly:

- 1) Silage storage: in a concrete-lined bunker silo, in an unlined trench silo, or in individual bags.
- 2) General housing conditions: ventilation and cleaning, including cleaning of walkways, stalls/bedding, and animals.
- 3) Milking system: milking parlor, pipeline tie-stall milking, or bucket tie-stall milking.
- 4) Milking procedure: identification of animals with mastitis, milking order (first: healthy cows, second: cows with subclinical mastitis, and finally, cows with clinical mastitis), predipping, forestripping, and checks to confirm that cows lie down after milking.

Somatic cell counts and bacterial counts (BC) in bulk-tank milk recorded for each farm as assessed monthly by the official dairy laboratory of the region.

Sample Collection and pH Determination

On each farm the following samples were collected in sterile containers following aseptic sampling procedures. 1) Bulk-tank milk was obtained at the upper opening of the tank after several minutes of stirring to ensure homogeneity; 2) pooled feces from 3 randomly selected, apparently healthy, lactating cows was taken directly from the rectum; 3) silage, both grass and corn, was sampled from the middle and lower parts of the silage storage container (bunker, trench, or bag).

All samples (98 milk samples, 97 fecal samples, and 83 silage samples) were transported to the laboratory

under refrigeration, and all analyses were performed within 24 h of sampling.

In addition, silage pH was determined at 3 locations in the silage container (upper, middle, lower) using a portable pH meter with penetration electrode (Hanna Instruments, Woonsocket, RI) with an accuracy of ± 0.02 pH units.

Isolation and Identification of *Listeria* Species

Listeria spp. were determined as described by Menéndez et al. (1997). Twenty-five grams of sample was transferred to a flask containing 225 mL of FDA *Listeria* enrichment broth (Merck, Darmstadt, Germany) and incubated for 48 h at 30°C. After enrichment, samples were plated onto PALCAM (polymixin B sulfate, acriflavine HCl, lithium chloride, ceftazidime, aesculin medium) agar (Merck) and incubated for 72 h at 37°C. Morphologically typical black colonies (tellurite-positive) with a black halo (esculin hydrolysis) were confirmed as *Listeria* on the basis of the catalase reaction with 3% H₂O₂, motility at 25°C, and sheep blood hemolysis, using the overlay technique and the API *Listeria* kit (bioMérieux, Marcy l'Etoile, France).

Data Analysis

Data were analyzed using Excel (Microsoft Corp., Redmond, WA) and the statistical software package SPSS 12.0 (SPSS Inc., Chicago, IL) with the aim of detecting differences between farms with *Listeria* spp. (or *L. monocytogenes*) in milk, and farms without *Listeria* in milk. Categorical variables were compared by χ^2 tests and quantitative variables by Student-Fischer *t*-tests (normally distributed data as indicated by Shapiro-Wilk test, $P \geq 0.05$) or Mann-Whitney *U*-tests (nonnormally distributed data). For identification of risk factors, odds ratios (OR) and corresponding confidence intervals (CI) were calculated. Statistical significance was indicated by $P \leq 0.05$ (Doménech, 2003).

RESULTS AND DISCUSSION

Listeria Prevalences in Milk

Listeria spp. were detected in 16.3% of bulk-tank milk samples (Table 1), a greater prevalence than the previously reported 4.1% in Finland (Husu, 1990), 8.3% in Ireland (Rea et al., 1992), 10.4% in the United States (Van Kessel et al., 2004), and 12.4% in Canada (Farber et al., 1988).

Listeria innocua was detected in milk from 7.1% of farms, similar to other studies that reported prevalence ranging from 2.3% in Sweden (Waak et al., 2002), 3.4% in Ireland (Rea et al., 1992), and 9.7% in Canada (Farber

Table 1. Prevalence of different *Listeria* species in bulk-tank milk, feces, and silage on 98 Galician dairy farms

<i>Listeria</i> species	Milk		Feces		Silage	
	Positive samples, n (%)	95% CI ¹	Positive samples, n (%)	95% CI	Positive samples, n (%)	95% CI
<i>L. monocytogenes</i>	6 (6.1)	1.4–10.8	9 (9.3)	3.5–15.1	5 (6.0)	0.9–11.1
<i>L. innocua</i>	7 (7.1)	2.0–12.2	22 (22.7)	14.4–31.0	16 (19.3)	10.8–27.8
<i>L. welshimeri</i>	1 (1.0)	0.0–5.6	4 (4.1)	1.1–10.2	4 (4.8)	1.2–11.9
<i>L. grayi</i>	2 (2.0)	0.3–7.2	4 (4.1)	1.1–10.2	2 (2.4)	0.3–8.4
<i>L. ivanovii</i>	—	—	1 (1.0)	0.0–6.5	—	—
<i>L. seeligeri</i>	—	—	—	—	1 (1.2)	0.0–6.5
Total	16 (16.3)	8.9–23.6	40 (41.2)	31.4–50.9	28 (33.7)	23.5–43.9

¹95% CI = confidence interval of odds ratio.

et al., 1988). *Listeria monocytogenes* was found in 6.1% of tanks, greater than the previously reported 1% in Sweden (Waak et al., 2002), 1.3% in Canada (Farber et al., 1988), 2.2% in Finland (Husu, 1990), 4.9% in Ireland (Rea et al., 1992), 4.1% in the United States (Rohrbach et al., 1992), and 4.6% in the United States (Jarayao and Henning, 2001), although similar to 6.5% in a recent study in the United States (Van Kessel et al., 2004). Hassan et al. (2000) analyzed milking equipment filters and found an *L. monocytogenes* prevalence of 12.6%. This was partly attributable to concentrations of the bacteria in the filter and dilution in the tanks, and to the lack of appropriate methods for isolating *Listeria* spp. from raw milk (Farber et al., 1988; Hassan et al., 2000). Reported prevalence of *Listeria* spp. varied greatly from study to study, possibly reflecting differences among regions or because of among-study differences in sample type (tank milk, filters) or analytical methods (Van Kessel et al., 2004). The prevalence of other *Listeria* species in milk samples was negligible: *L. grayi* in 2.0% of farms and *L. welshimeri* in 1.0%. These values approximate the 1.3% reported by Farber et al. (1988) for *L. welshimeri*.

Analysis of bulk-tank milk is important in view of the ability of *Listeria* spp. to survive at refrigeration temperatures and because of the techniques used to manufacture certain products from raw milk. For example, *L. monocytogenes* was detected in 6.4% (Rudolf and Scherer, 2001) and 4.9% (Pak et al., 2002) of samples of cheese produced from raw milk; in the former study, *L. innocua* was detected in 10.6% of samples and *L. seeligeri* in 1.2% (Rudolf and Scherer, 2001). In addition, *Listeria*-contaminated milk constitutes a risk for industrial dairy plants, for which raw milk is one of the principal sources of contamination (Menéndez et al., 1997).

Listeria Prevalence in Silage and Feces

Listeria spp. were detected in 32.2% of the 180 samples of silage and feces. This percentage is lower than the 77.8% obtained by Sanaa et al. (1996), but similar to the

30.6% obtained by Husu (1990). These values confirm the importance of environmental sources of *Listeria* spp. in dairy farms. In 7.2% of silage samples and 3.1% of fecal samples, more than 1 species of *Listeria* was detected. Reported prevalences of *Listeria* spp. in feces of healthy animals vary widely, apparently because of differences in sampling procedures and analytical methods (Husu, 1990).

Table 1 shows the prevalence of different *Listeria* spp. in milk, feces, and silage. *Listeria* spp. were detected in 41.2% of the 97 fecal samples analyzed, greater than the reported 8% in Sweden (Unnerstad et al., 2000) and 9.6% in Finland (Husu, 1990), but lower than the 81% from France (Sanaa et al., 1996). The presence of *Listeria* spp. in the feces of clinically healthy animals suggests that feces from such animals may be a source of contamination of milk (Unnerstad et al., 2000). We detected *Listeria innocua* in 22.7% of the samples, a greater prevalence than the 2% obtained in Sweden (Unnerstad et al., 2000) and the 4.6% in Finland (Husu, 1990). *Listeria monocytogenes* was present in 9.3% of our samples, similar to the 6% obtained by Unnerstad et al. (2000) and 6.7% obtained by Husu (1990), but lower than the value of 39% obtained by Sanaa et al. (1996). *Listeria welshimeri* was present in 4.1% of the samples, greater than the 0.2% obtained by Husu (1990). In addition, we detected *L. grayi* in 4.1% of our samples and *L. ivanovii* in 1.0%. Neither of these species was cited in the previous studies. *Listeria seeligeri* was not detected in fecal samples, as in previous studies (Unnerstad et al., 2000); Husu (1990) did detect this species, but only in 0.1% of samples.

We detected *Listeria* spp. in 33.7% of silage samples, a value intermediate between 22.7% in Finland (Husu, 1990) and 62% in France (Sanaa et al., 1996). *Listeria monocytogenes* was present in 6.0% of our silage samples, greater than the 3% obtained by Rea et al. (1992) in Ireland, but lower than the values of 16% obtained in Finland (Husu, 1990) and 39% in France (Sanaa et al., 1996). *Listeria innocua* was detected in 19.3% of our silage samples, greater than the 6% obtained by Rea et

Table 2. Associations between silage characteristics and presence of *Listeria* spp. in silage on 98 Galician dairy farms

Variable	Positive samples, n (%)	<i>P</i> -value ¹	Odds ratio	95% CI ²
pH ≥4.5				
No	1/16 (6.2)	0.05	6.28	0.77–51.16
Yes	18/61 (29.5)			
Storage				
Bunker silo	9/35 (25.7)	0.96	1.00	
Bag	3/12 (25.0)		0.97	
Unlined trench	8/28 (28.6)		1.11	

¹Significance ($P \leq 0.05$) of χ^2 test.²95% CI = confidence interval of odds ratio.

al. (1992). Other *Listeria* species were detected in silage with lower prevalence: 4.8% for *L. welshimeri*, 2.4% for *L. grayi*, and 1.2% for *L. seeligeri*.

The pH of silage samples from which *Listeria* spp. were isolated ranged from 4.47 to 6.97, a different range from those observed in other studies: 3.8 to 5.2 in Rea et al. (1992) and 5.78 to 5.89 in Ryser et al. (1997). The range obtained in the present study was more consistent with the lower pH limit of 4.5 for multiplication of *Listeria* spp. (Sanaa et al., 1993). This pH cut-off was selected in view of previous reports indicating that insufficient acidification of silage may favor the growth of *Listeria* spp., notably if pH is >4.0 (Sanaa and Ménard, 1994; Ryser et al., 1997) or 5.5 (Low and Donachie, 1997). The presence of *Listeria* spp. in silage was associated ($P = 0.05$) with silage pH (Table 2): when the pH was ≥4.5, the risk of presence of *Listeria* was increased (OR = 6.28). Silage storage method (concrete bunker silo, unlined trench, bagging) did not affect the presence of *Listeria* spp. (Table 2).

Relationship Between *Listeria* Presence in Milk, Silage, and Feces

Table 3 shows associations between the presence of *Listeria* spp. in milk, silage, and feces. In previous studies the proportion of farms with *Listeria* spp. present in all 3 sample types was as high as 50% (Rea et al., 1992). According to Sanaa and Ménard (1994), the presence of *L. monocytogenes* in silage, feces, or both increases the risk of presence in milk by a factor of 20, a finding we could not support.

Of the farms with *Listeria* spp. present in silage, 28.6% showed *Listeria* spp. in milk. Sanaa et al. (1993) reported that 8.5% of farms *Listeria* spp.-positive cases had silage with pH >4, and 68.7% did not use individual towels to clean udders before milking; we noted that the risk of contamination increased in farms with low-quality silage and with poor milking practices. Of the farms with *Listeria* spp. in feces, 24.3% showed *Listeria* spp. in milk,

close to the 24.4% obtained by Husu (1990). Other studies have likewise considered fecal material as a potential source of exogenous contamination of milk, principally due to poor hygiene practices during milking (Sanaa et al., 1996). Of the farms in the present study with *Listeria* spp. in silage, 38.1% showed *Listeria* spp. in feces, less than the 61.5% obtained by Husu (1990). This value (38.1% prevalence of *Listeria* spp. in feces, on farms with *Listeria* in silage) was similar to the value obtained for farms without *Listeria* in silage (39.3%; Table 3). Similarly to Husu (1990), we found no association ($P = 0.92$) between silage consumption and *Listeria* contamination of the feces of clinically healthy animals, although Ryser et al. (1997) found a similar association.

Risk Factors for the Presence of *Listeria* Species in Milk

Table 4 summarizes the results of associations between potential risk factors and the presence of *Listeria* spp. in milk. However, as pointed out by Hassan et al. (2001), interpretation of the effect of a given variable on the presence of *Listeria* should be based not solely on the presence or absence of a statistically significant association, but also on an understanding of the microorganism's biology.

With regard to general housing conditions, we considered the influence of ventilation and cleanliness on the presence of microorganisms in milk. We found that ventilation and cleanliness were not related ($P > 0.05$) to the presence of *Listeria* in milk. In 96.8% of the farms, the managers reported that teats were cleaned before placing the milking cups; despite this, *Listeria* spp. were detected in milk in 16.5% of farms. This might be because teat cleaning was not always adequately done, or because of the influence of other risk factors.

Protecting the milk from environmental contamination in stall milking systems is more difficult than in parlor milking systems, so that BC tends to be greater (Hassan et al., 2001), as was the case in the present

Table 3. Associations between presence of *Listeria* spp. in milk, silage, and feces on 98 Galician dairy farms

	<i>Listeria</i> -positive milk samples, n (%)	<i>P</i> -value ¹	Odds ratio	95% CI ²
<i>Listeria</i> spp. in silage		0.10	2.70	0.81–8.99
No	8/62 (12.9)			
Yes	6/21 (28.6)			
<i>Listeria</i> spp. in feces		0.10	2.43	0.82–7.23
No	7/60 (11.7)			
Yes	9/37 (24.3)			
	<i>Listeria</i> -positive fecal samples, n (%)			
<i>Listeria</i> spp. in silage		0.92	0.95	0.34–2.63
No	24/61 (39.3)			
Yes	8/21 (38.1)			

¹Significance ($P \leq 0.05$) of χ^2 test.

²95% CI = confidence interval of odds ratio.

study (pipeline average BC: 48×10^3 cfu/mL vs. parlor average: 32×10^3 cfu/mL). The risk of milk contamination by *Listeria* spp. was almost 3-fold greater on farms having a pipeline stall milking system ($P = 0.01$, OR = 2.87) than farms with parlor milking systems, as in previous studies (Rohrbach et al., 1992). Only 40.2% of farmers correctly identified cows with mastitis, and on these farms the probability of *Listeria* spp. in milk was lower (OR = 0.44; $P = 0.17$), but not different from farms with farmers who could not identify cows with mastitis. Al-

though *Listeria* spp. are isolated with rather low prevalence from the mammary glands (i.e., they are infrequently associated with mastitis; Rea et al., 1992; Rohrbach et al., 1992; Sanaa et al., 1996), the risk of milk contamination from cows with mastitis due to *Listeria* exists. In addition, following an appropriate order of milking reduces the likelihood of bacterial transmission between animals and of contamination of the milking equipment, and thus, reduces the probability of appearance of *Listeria* spp. in the bulk tank. The risk of contami-

Table 4. Associations between management practices and presence of *Listeria* spp. in bulk-tank milk on 98 Galician dairy farms

Variable	Positive samples, n (%)	<i>P</i> -value ¹	Odds ratio	95% CI ²
Stable management				
Ventilation				
Reduced	5/23 (21.7)	0.44	0.63	0.19–2.05
Good	11/74 (11.9)			
Cleanliness				
Poor	6/36 (16.7)	0.80	0.86	0.28–2.67
Good	9/61 (14.7)			
Milking				
Milking system				
Parlor	4/40 (10)	0.01	1.00	
Pipeline	12/42 (28.6)		2.87	
Bucket	0/15 (0)		0	
Identification of mastitic cows				
No	12/58 (20.7)	0.17	0.44	0.13–1.47
Yes	4/39 (10.3)			
Correct milking order				
No	8/25 (32)	0.01	0.26	0.08–0.81
Yes	7/65 (10.7)			
Forestripping				
No	2/20 (10)	0.37	2.03	0.42–9.79
Yes	14/76 (18.4)			
Check that cows lie down after milking				
No	8/64 (12.5)	0.18	2.13	0.69–6.56
Yes	7/30 (23.3)			

¹Significance ($P \leq 0.05$) of χ^2 test.

²95% CI = confidence interval of odds ratio.

nation of milk by *Listeria* was greater when a correct milking order was not followed ($P = 0.01$, $OR = 0.26$).

Association Among *Listeria* Contamination, SCC, and BC

Most of the farms had milk SCC $<400 \times 10^3$ cells/mL (83.9%), with 34.5% of farms having $<200 \times 10^3$ cell/mL. On 96.5% of farms, BC was $<100 \times 10^3$ cfu/mL, and on 42.4% of farms $<20 \times 10^3$ cfu/mL. Somatic cell counts and BC were not different ($P > 0.64$) in milk with *Listeria* than in milk without (SCC: 252×10^3 vs. 250×10^3 cells/mL; BC: 29×10^3 vs. 26×10^3 cfu/mL).

Considering SCC as a categorical variable ($<200 \times 10^3$ cells/mL, 200 to 400×10^3 cells/mL, and $>400 \times 10^3$ cells/mL), the prevalence of *Listeria* spp. in milk did not vary among the 3 categories ($P = 0.32$), as shown in other studies (Rohrbach et al., 1992; Hassan et al., 2000; Van Kessel et al., 2004). When BC was considered as a categorical variable ($<20 \times 10^3$ cfu/mL, 20 to 100×10^3 cfu/mL, and $>100 \times 10^3$ cfu/mL), the presence of *Listeria* spp. in milk again showed no significant variation among the 3 categories.

In conclusion, correct practices with respect to silage production and milking are essential for preventing introduction of *Listeria* into the herd, its spread within the herd, and its entry into milk. More studies are needed to clarify the significance of these risk factors, because only 1 sample was collected at each farm in the current study. The risk of contamination of milk by *Listeria* spp. increased when animals were fed low-quality silage, notably silage with $pH \geq 4.5$. The prevalence of *Listeria* species and *L. monocytogenes* in bulk-tank milk was similar to that reported in previous studies.

REFERENCES

- Cooper, J., and R. D. Walker. 1998. Listeriosis. Microbial food borne pathogens Vet. Clin. North Am. Food Anim. Pract. 14:113–125.
- Doménech, J. M. 2003. Fundamentos de Diseño y Estadística. UD 9: Comparación de dos proporciones. Medidas de asociación y efecto, UD 10: Relación entre dos variables categóricas: Pruebas de χ^2 , and UD 11. Comparación de dos medias: Pruebas *t*. Signo, Barcelona, Spain.
- Farber, J. M., G. W. Sanders, and S. A. Malcolm. 1988. The presence of *Listeria* spp. in raw milk in Ontario. Can. J. Microbiol. 34:95–100.
- Fenlon, D. R., and J. Wilson. 1989. The incidence of *L. monocytogenes* in raw milk from farm bulk tanks in North-East Scotland. J. Appl. Bacteriol. 66:191–196.
- Hassan, L., H. O. Mohammed, and P. L. McDonough. 2001. Farm-management and milking practices associated with the presence of *L. monocytogenes* in New York state dairy herds. Prev. Vet. Med. 51:63–73.
- Hassan, L., H. O. Mohammed, P. L. McDonough, and R. N. González. 2000. A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. J. Dairy Sci. 83:2441–2447.
- Husu, J. R. 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. J. Vet. Med. B. 37:276–282.
- Instituto Galego de Estatística. 2005. Explotación do Rexistro de Gando Bovino. http://www.ige.eu/ga/economicas/agro_pesca/rexistro_bovino/index.htm Accessed Jan. 17, 2005.
- Jarayao, B. M., and D. R. Henning. 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci. 84:2157–2162.
- Low, J. C., and W. Donachie. 1997. A review of *Listeria monocytogenes* and listeriosis. Vet. J. 153:9–29.
- Menéndez, S., M. R. Godínez, J. L. Rodríguez-Otero, and J. A. Centeno. 1997. Removal of *Listeria* spp. in a cheese factory. J. Food Safety 17:133–139.
- Pak, S. I., U. Spahr, T. Jemmi, and M. D. Salman. 2002. Risk factors for *L. monocytogenes* contamination of dairy products in Switzerland, 1990–1999. Prev. Vet. Med. 53:55–65.
- Rea, M. C., T. M. Cogan, and S. Tobin. 1992. Incidence of pathogenic bacteria in raw milk in Ireland. J. Appl. Bacteriol. 73:331–336.
- Rohrbach, B., F. A. Draughon, P. M. Davidson, and S. P. Oliver. 1992. Prevalence of *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica*, and *Salmonella* in bulk tank milk: Risk factors and risk of human exposure. J. Food Prot. 55:93–97.
- Rudolf, M., and S. Scherer. 2001. High incidence of *Listeria monocytogenes* in European red smear cheese. Int. J. Food Microbiol. 63:91–98.
- Ryser, E., S. M. Arimi, and C. W. Donnelly. 1997. Effects of pH on distribution of *Listeria* ribotypes in corn, hay and grass silage. Appl. Environ. Microbiol. 63:3695–3697.
- Sanaa, M., A. Audurier, B. Poutrel, J. L. Menard, and F. Serieys. 1996. Origin of bovine raw milk contamination by *Listeria monocytogenes*. Pages 84–88 in Symposium on Bacteriological Quality of Raw Milk, IDF. Austria. Int. Dairy Fed., Brussels, Belgium.
- Sanaa, M., and J. L. Ménard. 1994. Contamination du lait cru par *L. monocytogenes*: Origines, facteurs de risque, prévention. Rec. Méd. Vet. 170:437–442.
- Sanaa, M., B. Poutrel, J. L. Ménard, and F. Serieys. 1993. Risk factors associated with contamination of raw milk by *L. monocytogenes* in dairy farms. J. Dairy Sci. 76:2891–2898.
- Unnerstad, H., A. Romell, H. Ericsson, M. L. Danielsson-Tham, and W. Tham. 2000. *Listeria monocytogenes* in faeces from clinically healthy dairy cows in Sweden. Acta Vet. Scand. 41:167–171.
- Van Kessel, J. S., J. S. Karns, L. Gorski, B. J. McCluskey, and M. L. Perdue. 2004. Prevalence of *Salmonella*, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. J. Dairy Sci. 87:2822–2830.
- Vázquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14:584–640.
- Waak, E., W. Tham, and M. L. Danielsson-Tham. 2002. Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Appl. Environ. Microbiol. 68:3366–3370.
- WHO. 2002. Food safety and foodborne illness. <http://www-who.int/mediacentre/factsheets/fs237/en/> Accessed May 12, 2005.
- Wiedmann, M. 2003. An integrated science based approach to dairy food safety: *Listeria monocytogenes* as a model system. J. Dairy Sci. 86:1865–1875.
- Winter, P., F. Schilcher, Z. Bagò, D. Schoder, M. Egerbacher, W. Baumgartner, and W. Wagner. 2004. Clinical and histopathological aspects of naturally occurring mastitis caused by *Listeria monocytogenes* in cattle and ewes. J. Vet. Med. 51:176–179.